CLAIMS

1. A composition comprising: (1) a polynucleotide target; (2) a first target-hybridizing probe which comprises a target binding sequence (P1-DNA) which hybridizes to a strand of said target polynucleotide and a probe binding sequence (P1-P); and (3) a second target-hybridizing probe which comprises a target binding sequence (P2-DNA) which hybridizes, in close proximity, to said strand of said target polynucleotide and a probe binding sequence (P2-P).

2. The composition of claim 1, further comprising a non-target-hybridizing probe 3 labeled with label A and a non-target-hybridizing probe 4 labeled with label B, wherein said probe 3 hybridize to said P1-P sequence and said probe 4 hybridizes to said P2-P sequence.

3. The composition of claim 2, wherein said label A interacts with said label B to generate a signal indicative of an amount of said target polynucleotide.

4. A composition comprising: (1) a target polynucleotide; (2) a first target-hybridizing probe comprises a target binding sequence (P1-DNA) which is complementary to a first sequence on a strand of said target polynucleotide and a probe-binding sequence (P1-P); (3) a second target-hybridizing probe comprises a target binding sequence (P2-DNA) which is complementary to a second sequence on said strand of said target polynucleotide and a probe-binding sequence (P2-P); (4) a non-target-hybridizing probe 3 labeled with label A; and (5) a non-target-hybridizing probe 4 labeled with label B, wherein said P1-P sequence is complementary to probe 3 and said P2-P sequence is complementary to probe 4, and said label A interacts with said label B to generate a signal indicative of an amount of said target polynucleotide.

5. The composition of claim 4, wherein said first target-hybridizing probe and said second target-hybridizing probe hybridize to a same strand of said target polynucleotide in close proximity.

6. The composition of claim 5, wherein said probe 3 hybridizes to said P1-P sequence and said probe 4 hybridizes to said P2-P sequence.

7. The composition of claim 2 or 4, wherein said label A and label B are members of a pair of interactive labels.

8. The composition of claim 1 or 5, wherein said target binding sequence is at 5' of said probe binding in said probe 1, while said target binding sequence is at 3' of said probe binding sequence in said probe 2.

9. The composition of claim 8, wherein said label A and label B are fluorescent dyes.

10. The composition of claim 9, wherein said label A and label B are a donor-acceptor pair which interact with each other to generate a signal by fluorescent resonance energy transfer (FRET).

11. The composition of claim 10, wherein said donor-acceptor pair is a FAM/ROX pair.

12. The composition of claim 10, wherein said acceptor is a dark quencher.

13. The composition of claim 4, wherein said probe 3 or 4 shares no homology to any polynucleotide isolated from a sample containing said target polynucleotide.

14. The composition of claim 4, wherein said probe 3 is labeled at its 3' terminal and said probe 4 is labeled at its 5' terminal.

15. The composition of claim 4, wherein the 3' terminal of a probe is modified to prevent probe extension.

16. The composition of claim 15, wherein said 3' terminal of a probe is phosphorylated.

17. The composition of claim 5, wherein said probe 3 has a higher melting point (Tm) than said P1-DNA sequence, and said probe 4 has a higher Tm than said P2-DNA sequence.

18. The composition of claim 1 or 4, further comprising a forward and a reverse primer for the amplification of said target polynucleotide.

19. The composition of claim 18, wherein when said P1-DNA and P2-DNA bind to the strand amplified by said reverse primer, the amount of said forward primer to the amount of said reverse primer in said composition is 1:5; and when said P1-DNA and P2-DNA binds to the strand amplified by said forward primer and the amount of said forward primer to the amount of said reverse primer is 5:1.

20. The composition of claim 1 or 4, further comprising a control polynucleotide.

21. A kit comprising (1) a polynucleotide target; (2) a first target-hybridizing probe which comprises a target binding sequence (P1-DNA) which hybridizes to a strand of said target polynucleotide and a probe binding sequence (P1-P); and (3) a second target-hybridizing probe which comprises a target binding sequence (P2-DNA) which hybridizes, in close proximity, to said strand of said target polynucleotide and a probe binding sequence (P2-P) and (3) packaging materials therefor.

22. The kit of claim 21, further comprising a non-target-hybridizing probe 3 labeled with label A and a non-target-hybridizing probe 4 labeled with label B, wherein said probe 3 hybridize to said P1-P sequence and said probe 4 hybridizes to said P2-P sequence.

23. The kit of claim 22, wherein said label A interacts with said label B to generate a signal indicative of an amount of said target polynucleotide.

24. A kit comprising: (1) a target polynucleotide; (2) a first target-hybridizing probe comprises a target binding sequence (P1-DNA) which is complementary to a first sequence on a strand of said target polynucleotide and a probe-binding sequence (P1-P); (3) a second target-hybridizing probe comprises a target binding sequence (P2-DNA) which is complementary to a second sequence on said strand of said target polynucleotide and a probe-binding sequence (P2-P); (4) a non-target-hybridizing probe 3 labeled with label A; (5) a non-target-hybridizing probe 4

labeled with label B, wherein said P1-P sequence is complementary to probe 3 and said P2-P sequence is complementary to probe 4, and said label A interacts with said label B to generate a signal indicative of an amount of said target polynucleotide; and (6) packaging materials therefor.

25. The kit of claim 24, wherein said first target-hybridizing probe and said second target-hybridizing probe hybridize to a same strand of said target polynucleotide in close proximity.

26. The kit of claim 25, wherein said probe 3 hybridizes to said P1-P sequence and said probe 4 hybridizes to said P2-P sequence.

27. The kit of claim 22 or 24, wherein said label A and label B are members of a pair of interactive labels.

28. The kit of claim 27, wherein said label A and label B are fluorescent dyes.

29. The kit of claim 28, wherein said label A and label B are a donor-acceptor pair which interact with each other to generate a signal by fluorescent resonance energy transfer (FRET).

30. The kit of claim 29, wherein said donor-acceptor pair is a FAM/ROX pair.

31. The kit of claim 29, wherein said acceptor is a dark quencher.

32. The kit of claim 24, wherein said probe 3 or 4 shares no homology to any polynucleotide isolated from a sample containing said target polynucleotide.

33. The kit of claim 24, wherein said probe 3 is labeled at its 3' terminal and said probe 4 is labeled at its 5' terminal.

34. The kit of claim 24, wherein the 3' terminal of a probe is modified to prevent probe extension.

35. The kit of claim 29, wherein said 3' terminal of a probe is phosphorylated.

36. The kit of claim 25, wherein said probe 3 has a higher melting point (Tm) than said P1-DNA sequence, and said probe 4 has a higher Tm than said P2-DNA sequence.

37. The kit of claim 21 or 24, further comprising a forward and a reverse primer for the amplification of said target polynucleotide.

38. The kit of claim 37, wherein when said P1-DNA and P2-DNA binds to the strand amplified by said reverse primer, the amount of said forward primer to the amount of said reverse primer in said kit is 1:5; and when said P1-DNA and P2-DNA binds to the strand amplified by said forward primer and the amount of said forward primer to the amount of said reverse primer is 5:1.

39. The kit of claim 21 or 24, further comprising a control polynucleotide.

40. The kit of claim 21 or 25, wherein said target binding sequence is at 5' of said probe binding in said probe 1, while said target binding sequence is at 3' of said probe binding in said probe 2.

41. A method for detecting the amount of a target polynucleotide, comprising:

(a) adding to said target polynucleotide: (1) a target-hybridizing probe 1 comprising a target binding sequence (P1-DNA) which hybridizes to one strand of said target polynucleotide and a probe binding sequence (P1-P), (2) a target-hybridizing probe 2 comprising a target binding sequence (P2-DNA) which hybridizes, in close proximity, to the same strand of said target polynucleotide and a probe binding sequence (P2-P); (3) a non-target-hybridizing probe 3 labeled with label A which hybridizes to said P1-P sequence, and (4) a non-target-hybridizing probe 4 labeled with label B which hybridizes to said P2-P sequence, wherein said addition permits said label A to interact with said label B to generate a detectable signal; and

(b) detecting said generated signal as indicative of the amount of said polynucleotide.

42. A method for detecting the amount of a target polynucleotide in an amplification reaction mixture, comprising

(a) adding to said amplification reaction mixture: (1) a target-hybridizing probe 1 comprising a target binding sequence (P1-DNA) which hybridizes to one strand of said target polynucleotide and a probe binding sequence (P1-P), (2) a target-hybridizing probe 2 comprising a target binding sequence (P2-DNA) which hybridizes, in close proximity, to the same strand of said target polynucleotide and a probe binding sequence (P2-P), (3) a non-target-hybridizing probe 3 labeled with label A which hybridizes to said P1-P sequence, and (4) a non-target-hybridizing probe 4 labeled with label B which hybridizes to said P2-P sequence, wherein said addition permits said label A to interact with said label B to generate a signal; and

(b) detecting said generated signal as indicative of the amount of said polynucleotide.

43. The method of claim 41 or 42, wherein said label A and label B are members of a pair of interactive labels.

44. The method of claim 43, wherein said label A and label B are fluorescent dyes.

45. The method of claim 44, wherein said label A and label B are a donor-acceptor pair which interact with each other to generate a signal by fluorescent resonance energy transfer (FRET).

46. The method of claim 45, wherein said donor-acceptor pair is a FAM/ROX pair.

47. The method of claim 46, wherein said acceptor is a dark quencher.

48. The method of claim 41 or 42, wherein said probe 3 or 4 shares no homology to any polynucleotide isolated from a sample containing said target polynucleotide.

49. The method of claim 41 or 42, wherein said probe 3 is labeled at its 3' terminal and said probe 4 is labeled at its 5' terminal.

50. The method of claim 41 or 42, wherein the 3' terminal of a probe is modified to prevent probe extension.

51. The method of claim 50, wherein said 3' terminal of a probe is phosphorylated.

52. The method claim 41 or 42, wherein said target binding sequence is at 5' of said probe binding in said probe 1, while said target binding sequence is at 3' of said probe binding in said probe 2.

53. The method of claim 41 or 42, wherein said probe 3 has a higher melting point (Tm) than said P1-DNA sequence, and said probe 4 has a higher Tm than said P2-DNA sequence.

54. The method of claim 42, wherein when said P1-DNA and P2-DNA binds to the strand amplified by a reverse primer, the amount of a forward primer to the amount of said reverse primer in said reaction mixture is 1:5; and when said P1-DNA and P2-DNA binds to the strand amplified by a forward primer and the amount of said forward primer to the amount of a reverse primer is 5:1.

55. The method of claim 42, wherein said amplification reaction is a polymerase chain reaction (PCR).

56. The method of claim 55, wherein said generated detectable signal is detected at the end of two or more PCR cycle.

57. The method of claim 41 or 42, wherein said generated signal is detected and compared with signals generated by one or more reference containing a known amount of said target polynucleotide for the determination of the amount of said target polynucleotide.